MINIREVIEW

Herbogenomics: From Traditional Chinese Medicine to Novel Therapeutics

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Traditional Chinese medicine (TCM) has a long history of development and application and has demonstrated on evidence basis its efficacy in the treatment of many diseases affecting multiple organ systems. In particular, TCM is effective in the prevention and treatment of chronic diseases and metabolic syndromes. However, the value of TCM has not been fully recognized worldwide due to the lack of definitive information of active ingredients in almost any TCM preparation. Novel functional genomics and proteomics approaches provide alternate perspectives on the mechanism of action of TCM. The target molecules on which TCM either activates or inactivates can be identified by functional genomics and proteomics, thus the affected critical signaling pathway cascades leading to effective recovery of chronic diseases can be studied. Several TCM preparations have been available for the treatment of liver fibrosis and cirrhosis, even advanced liver cirrhosis that has been shown to be irreversible and has no US-FDA approved therapy. In the TCM-treated livers with fibrosis and cirrhosis, some critical molecules that are significantly involved in the recovery can be identified through functional genomics and proteomics studies. These molecules become novel targets for drug discovery and development and candidates for the development of gene therapy. Gene therapy developed based on this strategy for the treatment of advanced liver fibrosis and cirrhosis in animal models has obtained promising results. This process thus establishes a herbogenomics approach to understand mechanisms of action of TCM and to identify effective molecular targets for the discovery and development of novel therapeutics. Exp Biol Med 233:1059–1065, 2008

Key words: TCM; gene therapy; personalized medicine; proteomics, genomics; liver fibrosis; cirrhosis

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Introduction

Herbogenomics is defined as the analysis of the biological effect on the target organs of a particular herbal medicine preparation through a profiling of the affected genomic and proteomic alterations. By doing so, herbogenomics provides a mechanistic understanding of the efficacy and toxicity of a particular herbal medicine preparation. The technology of herbogenomics has been under exponential development and increasingly applied in the basic research and clinical studies of traditional Chinese medicine (TCM).

TCM, referred to as the traditional Chinese herbal medicine preparation, is of a unique value in the treatment of multiple organ system disorders, in particular chronic diseases and metabolic syndromes. TCM is effective in some cases such as advanced liver fibrosis or liver cirrhosis (1–10), to which there is no US-FDA approved therapy. However, there are several obstacles for the advancement and the application of TCM in patients worldwide. The key issue is that the active ingredients in TCM are unknown. This problem is further amplified to several related shortcomings, including the unstable feature of the therapeutic efficacy of different preparations of the same TCM, unknown toxicity of TCM preparations, and the lack of Western standardized clinical trials (1, 2, 11, 12).

The uniqueness of TCM in the treatment of certain chronic diseases and the obstacles for its acceptance by healthcare providers and patients constitute a dilemma of modern medicine. However, the increasing recognition of the value of complementary and alternative medicine (CAM) in the healthcare market has dictated a new demand of novel insights into TCM. The advances in molecular medicine and biotechnology provide new tools to alternative approaches to the understanding of TCM action and mechanism. Herbogenomics thus becomes practically available. The value of herbogenomics in the understanding

of TCM has been recognized in basic and clinical studies of TCM (13), and would be further explored along with the increasing worldwide distribution of TCM.

There are at least two major areas of modern medicine that can be enriched by herbogenomics. First, it would greatly help understand the mechanism of action of TCM as well as the adverse effect of TCM on target organs so that further regulation of worldwide distribution of TCM can be developed. Second, the discovery and development of new drugs for the treatment of currently untreatable diseases by Western medicine would benefit from the finding of herbogenomics. Some new molecular targets and signaling pathways can be identified through the herbogenomic analysis of target organs, which would become a platform for the development of novel therapies including new drugs and gene therapy products.

In light of the increasing recognition of TCM in modern medicine, this review will highlight some unique values of TCM in the treatment of chronic disease such as advanced liver fibrosis and cirrhosis and briefly summarize the current state of TCM development. With the understanding of the current status of TCM in modern medicine, a novel herbogenomics as an alternative approach will be presented and the bonus value of herbogenomics in the drug discovery and development and in the advance of molecular therapy for advanced liver fibrosis to which there is currently no effective therapy will be elucidated.

TCM in the Treatment of Advanced Liver Fibrosis and Cirrhosis. The efficacy of TCM in the treatment of several chronic and metabolic diseases, such as advanced liver fibrosis and cirrhosis, has been observed clinically and explored in experimental studies. However, this value of TCM has not been fully recognized worldwide. There are several reasons for this gap, but two factors play a critical and determinant role.

First, the TCM clinical practice follows a different standard from the Western medicine. The most distinguished TCM practice is that patients are treated with a particular basic TCM preparation but different variations based upon the individual diagnosis. This takes advantage of the nature of TCM preparation, which is a mixture of several ingredients. The quality and quantity composition of each ingredient in a TCM preparation is modified based upon the diagnosis of the individual patient. This practice realizes not only the existence of a particular disease, but also the stage of disease progression and the affected secondary organ systems. This personalized medicine has been the standard practice of TCM since its invention about 3,100 years ago (14). "Personalized medicine" has recently become realized in Western medicine. This practice is based upon the understanding of pharmacogenomics and pharmacogenetics of individual patients.

Second, the documentations of the efficacy and toxicity of many effective TCM preparations have been mostly in Chinese or other Asian languages. The translation of these wealthy and valuable documents to Western languages including English has not been undertaken. This problem is the derivative of the different standard of practice between TCM and Western medicine. Without knowing the active ingredients in the TCM mixture, the publication of TCM basic and clinical studies in mainstay medical journals is difficult.

Liver fibrosis is characterized by an excessive deposition of extracellular matrix (ECM) proteins, which accompanies altered compositions of the ECM proteins, such as a significant increase in collagens in the ECM (15). Progression of liver fibrosis leads to liver architectural distortion resulting from dense bands of collagens that link vascular structures and surround islands of regenerating parenchymal cells, the nodule formation, which is characteristic of liver cirrhosis (15, 16), the end stage of fibrosis. Advanced liver fibrosis or liver cirrhosis leads to severe pathological disturbances such as further remodeling of the liver architecture and the hepatic functional failure (15–17). Liver cirrhosis was viewed as an irreversible condition for which therapy could offer only transient symptomatic relief, and patients with liver cirrhosis would rely on only transplantation to improve survival.

Hepatic fibrogenesis is the consequence of a sustained wound healing response to chronic liver injury from a diversity of etiologies (17). Virtually any chronic toxic insult such as viral infection, alcoholic liver injury, drug toxicity, cholestatic and metabolic disturbances, or exposures to toxic environmental substances can lead to liver fibrosis and cirrhosis (18–22). Therefore, treatment targeting the etiology of liver injury is an effective approach to inhibit progression of liver fibrosis. Recent clinical and animal model studies have shown that advanced liver fibrosis and cirrhosis can be reversed under certain conditions (23–25). Interestingly, in TCM practice, advanced liver fibrosis and cirrhosis has long been considered reversible.

Han-Dan-Gan-Le (HDGL), a Chinese medicine preparation composed of *Salvia miltiorhiza Bge*, *Paeonia Lactflora Pall*, *Astragalus*, *Stephania tetrandra*, and dried leaves of *Ginkgo biloba*, has been used to treat human liver fibrosis and cirrhosis with a high efficacy for years in China. This herbal medicine preparation has been used to treat a large volume of patients with advanced liver fibrosis and cirrhosis induced by toxic exposures (3), HBV infection (4–6), and chronic liver metabolic disorders (7). Clinical experience has proven high efficacy and low toxicity of HDGL in the treatment of advanced liver cirrhosis (8). Animal studies have duplicated the clinical observation. In a rat model of carbon tetrachloride-induced liver fibrosis, HDGL has been shown both to prevent liver fibrosis if given simultaneously with carbon tetrachloride exposure (9) and to reverse the condition if given after the liver fibrosis has been established (10), although the mechanism of action of HDGL remains elusive.

Current State of TCM in Modern Medicine. The major strength of the TCM preparation, the mixture of several ingredients, is also its major weakness. The strength

is that in this mixture the addition, reduction or quantitative modification of an ingredient can be easily achieved and accommodated to the highest efficacy of therapy for a particular patient. The weakness is that the exact active ingredients in any of the TCM mixture are unknown; therefore, the application of the Western standardized procedure for preparation and analysis of drugs to TCM becomes a very difficult task.

During the last four decades, many efforts have been focused on the isolation and purification of active ingredients in TCM preparations for the purpose of modernization of TCM. However, isolation and purification of active ingredients in TCM for the understanding of the mechanism of action, efficacy and toxicity have proven extremely difficult. There are more than a thousand different ingredients in any TCM mixture preparation. It is this character of mixture that makes TCM unique and effective in the treatment of some chronic and metabolic diseases. The quantitative composition of each ingredient in TCM is expected to be different and some trace amount may play a critical role in the efficacy of a TCM. Analysis both qualitatively and quantitatively of these trace components in TCM is extremely difficult. If we cannot completely identify the composition of a TCM preparation, how would we reconstitute a naturally occurred medicine? A good example is the effort we have made to the analysis of green tea, in which we have realized that there are thousands of active ingredients and only the naturally occurred mixture of green tea has its recognized benefits to health. However, the isolation and purification of certain ingredients in green tea for the purpose of developing new drugs or supplements is a different action from that of reconstituting the composition of green tea.

Modification of its composition based upon a patient's condition is an advantage of the TCM preparation; however, it is also a concern of inconsistency and unstableness of a particular TCM preparation. The TCM practitioners believe that each patient needs to be treated differently so that the composition of a particular TCM needs to be constituted according to individual diagnosis and constantly modified during the course of treatment in order to reach the highest efficacy of therapy. It is difficult to follow a standard placebo-controlled clinical trial with personalized medicine. However, in the modern era of medical practice, the application of personalized medicine has been recommended. The difference between the TCM and modern personalized medicine is that in TCM the personalized medicine is based upon the experience of a TCM practitioner, but modern medicine is based upon advanced biotechnology.

Toxicity of a particular TCM preparation is difficult to evaluate, but the lack of a comprehensive understanding of a TCM preparation may cause a mislabeling of an active ingredient as a toxic component. This lack of a comprehensive understanding of the relationship between a TCM composition and the therapeutic efficacy makes another difficulty for the advancement of TCM. For instance, there are several metal compositions in some TCM preparations and these metals may play a major role in the therapeutic action of a TCM. However, the level of the metal content is often several thousands folds higher than what is considered safe for environmental exposure to humans, so that it is labeled as an unacceptable contamination. But removal of these metals from some TCM preparations significantly reduces the therapeutic efficacy. This has been demonstrated in some TCM preparations containing cinnabar (containing a high level of mercury sulfide).

Cinnabar has been used for more than 2,000 years in TCM and Indian Ayurvedic medicine (26, 27). It has been documented that the inclusion of cinnabar in a very effective TCM preparation, An-Gong-Niu-Huang-Wan, is essential (28) or beneficial (29), although some studies also suggest it is not important (30). The content of mercury in these TCM preparations is about thousands folds higher than what is considered safe in the experimental exposure, so that many cinnabar-containing TCM have been banned (31). However, it is possible that mercury in its sulfide salt form is less toxic (31) but essentially involved in the active ingredients of some TCM preparations. Without a comprehensive understanding of the relationship between the cinnabar therapeutic and toxic relevance, the advancement in the unique and effective cinnabar-containing TCM is very challengeable.

Arsenic, unlike mercury, has been accepted as an active ingredient in several TCM preparations. Arsenic is an environmental toxicant, which has been recognized for almost two centuries (32). Many experimental and epidemiological studies have suggested that arsenic is a potent cocarcinogen that is involved in several human malignancies including skin and lung cancers (33–35). Interestingly, arsenic compounds have been used for medication for human diseases for a long time (36, 37). In TCM, arsenic trioxide had been used to treat psoriasis, syphilis and rheumatosis (37). A striking development in using arsenic for medication is its application in the treatment of acute promyelocytic leukemia (APL). It was found that arsenic trioxide is very effective in the treatment of APL and the clinical complete remission rate reached from 65% to 84% (38). The US-FDA approved the Trisenox brand of arsenic trioxide for injection for the treatment of relapsed and refractory APL in the United States in 2000. The dose regiment of arsenic trioxide has been reported to be 10 mg/d by intravenous infusion for 28 to 60 days (39). This level is many thousands folds higher than what is considered safe for environmental exposure to humans. However, arsenic trioxide is applied to APL patients, indicating the response of the patient population to arsenic trioxide is different from that of healthy populations. In this context, the several metal compositions in TCM preparations may well be the active ingredients for the therapeutic effect, which demands a higher level for the efficacy. Therefore, the lack of a comprehensive understanding of TCM therapeutic efficacy and toxicity makes the active ingredients and toxic

components indistinguishable, being an important problem in the advancement of TCM.

Herbogenomics in the Understanding of TCM Action. The uniqueness and effectiveness of TCM in the treatment of some chronic diseases and metabolic syndromes would have a significant impact on people's health worldwide. While current efforts of identifying active ingredients for the understanding of the mechanism of action of TCM remain active, alternative approaches are also being explored.

The application of an herbogenomics approach to the understanding of the efficacy and toxicity of a particular TCM will provide novel insights into the mechanism of action of TCM. This approach will validate the effectiveness of TCM with a sound scientific reasoning process. This approach combines the unique effectiveness of TCM with the state-of-the-art technologies of proteomics and genomics, constituting a novel platform of TCM study. In any effective treatment of a particular disease with TCM, the ultimate effecters for the efficacy have to be the molecular alteration in the forms of differential regulation of gene expression and/or post-translational modifications of proteins. Such molecular alterations can be revealed through microarray of mRNAs and profiling of proteins. The information regarding up-regulation or down-regulation of some genes or activation or deactivation of some regulatory proteins will provide new insights into the mode of action of a TCM preparation. Further identification and validation of the effect of these molecules on disease progression or regression will elucidate the affected targets that mediate the action of TCM.

Based on this understanding, a novel platform for the understanding of mechanisms of action of TCM and the link of this understanding to new drug discovery and development and molecular therapy is proposed. The scheme presented in Figure 1 describes this novel platform. There are effective TCM preparations that can cure several chronic diseases such as liver fibrosis and cirrhosis (1–10), viral cardiomyopathy (40–43), inflammatory bowel disease (44, 45), diabetic nephropathy (46–49), and rheumatoid arthritis (50, 51). However, these TCM preparations are not scientifically proven because of unknown active ingredients. This novel platform is to focus on the therapeutic effects of TCM on human patients and recapitulation of the disease regression effects of TCM in animal disease models, instead of on identification of the active ingredients. The reason for this approach is that the unique effect of TCM-induced recovery of chronic diseases provides an exceptional opportunity to determine molecular and cellular targets that are involved in the disease regression process. The analysis of target cells and molecules provides scientific validation of TCM. In addition, new therapeutic products for disease regression can be discovered and developed based upon the analysis of the target effect of TCM. Such a validation procedure has been applied to a TCM preparation, Han-Dan-Gan-Le, which has been used in China for the treatment of advanced liver fibrosis and cirrhosis (3–7).

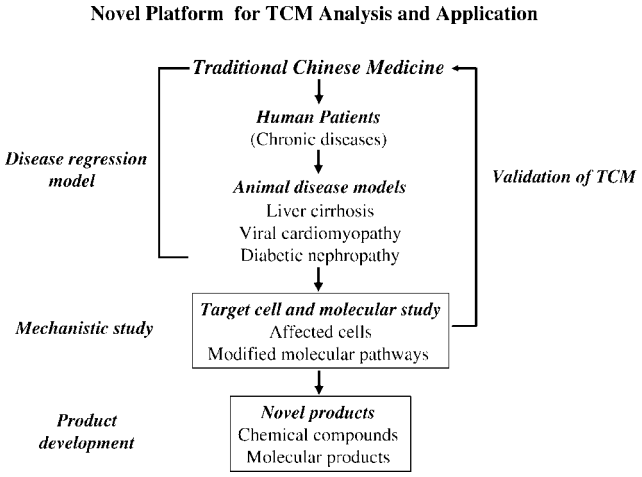

Figure 1. Novel platform for TCM analysis and application. Clinically proven effective TCM preparations can be used in animal models of chronic diseases to define mechanisms of action of the TCM preparation. From the mechanistic studies, the cellular and molecular targets that play a crucial role in the recovery of chronic diseases can be identified, which become the basis for the discovery and development of novel therapeutics.

Han-Dan-Gan-Le (HDGL) is a TCM preparation composed of extracts from 5 different herbals of medication indication, as discussed above (7, 9, 10), and is highly effective in the treatment of advanced liver fibrosis or cirrhosis (3–7). This TCM preparation has been used for a long time in China, but the mechanism of action has not been understood. Efforts have been made to provide mechanistic insights into the action of HDGL. The analysis using genomic and proteomic techniques have identified that HDGL up-regulates several critical proteins that are involved in the regulation of collagen synthesis and degradation and the overall action of HDGL is to promote fibrolysis in the liver with advanced fibrosis (10). This understanding helps the explanation of the observed reversal of advanced liver fibrosis in humans and animal models (7, 9, 10), and also helps the development of gene therapy for advanced liver fibrosis (52).

Herbogenomics in Drug Discovery and Development. Natural products have historically served as an invaluable source of pharmaceutical discovery and development. However, the emphasis in the pharmaceutical industry on the discovery of natural products has declined during the last decade. The introduction of high-throughput screening technique made pharmaceutical companies switch their screening targets from natural products libraries to screen friendly synthetic chemical libraries. Furthermore, the advance in molecular and cellular biology made the design and analysis of structure-activity more emphasized on the cellular and molecular targets, which in combination with the development of combinatorial chemistry has increased the emphasis on more drug-like screening libraries. This

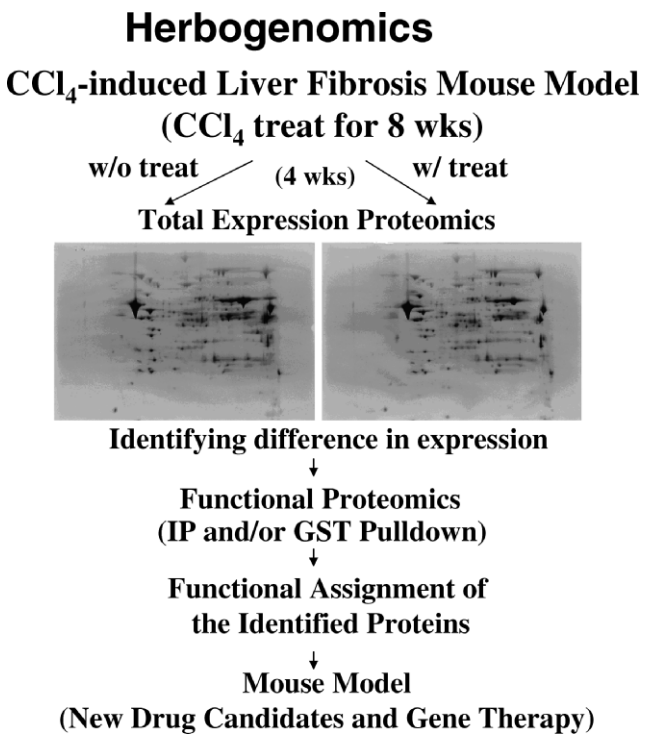

Figure 2. Discovery and development of new drugs and molecular therapy through herbogenomics. The example used is HDGL induced regression of advanced liver fibrosis induced by carbon tetrachloride. Expression proteomics can identify the alterations in the profile of protein expression and post-translational modification in response to HDGL treatment. Further analysis using functional proteomics will help define the function of the identified proteins. These proteins then serve as targets for the discovery and development of new drugs and gene therapy, which will be tested in animal models of chronic diseases.

shift would provide a good opportunity for the application of herbogenomics in drug discovery and development.

As depicted in Figure 2, the analysis using proteomics techniques will help define the target molecules that play a critical role in the regression of advanced liver fibrosis in response to the therapeutic effect of TCM preparation. In this particular case, advanced liver fibrosis was induced by treating mice with carbon tetrachloride for 8 weeks. Upon cessation of the treatment, liver fibrosis would not be reversed within a period of up to 12 weeks. In contrast, liver fibrosis would be reversible upon cessation of the treatment in mice treated with carbon tetrachloride for 4 weeks (52). After the treatment with carbon tetrachloride for 8 weeks, the mice were treated with HDGL for 4 weeks. This treatment resulted in regression of the advanced liver fibrosis. Liver tissues from mice treated with HDGL for 4 weeks after treatment with carbon tetrachloride for 8 weeks, along with the tissue from mice treated with carbon tetrachloride for 8 weeks followed by saline treatment for additional 4 weeks as control, were subjected to expressional proteomics analysis.

The change in the profile of proteins that were potentially involved in the regression of advanced liver fibrosis was then detected by a 2D gel electrophoresis and individual proteins were identified through mass spectrometry analysis. These proteins include those that are involved in the production and degradation of extracellular matrix metalloproteins or collagens. Some proteins are also involved in the activation and propagation of hepatic stellate cells. These proteins are critically involved in the initiation and progression of liver fibrosis. However, an important question is: What is the primary target that is sensitive to HDGL and plays a regulatory role in the regression of advanced liver fibrosis?

A functional proteomics analysis was applied to define the relationship between the proteins whose profiles changed in response to HDGL treatment. This analysis helps define the relationship between different regulatory proteins and critical molecules that can initiate the cascade of regression of advanced liver fibrosis. These targets identified through the functional proteomics analysis then serve as novel targets for new drug development.

These new molecular targets are further validated using animal models of advanced liver fibrosis by specific gene transfection or deletion. The validated targets then become a new foundation for drug discovery and development and gene therapy. The drug candidates designed or screened based upon these new targets are then tested using animal disease models. This herbogenomics approach thus provides a novel platform for new drug discovery and development and ascribes natural protectants to a new role in the pharmaceutical industry.

Herbogenomics and Gene Therapy. Herbogenomics analysis of HDGL-induced regression of advanced liver fibrosis, as shown in Figure 2, revealed the profile of alterations in protein expression and post-translational modifications. Among the proteins that are significantly modified by HDGL is metallothionein (MT). MT is an important zinc-binding protein and is involved in zinc metabolism and homeostasis (53–55). The interaction of MT with a number of oxidants causes zinc release from the protein (54, 55), and the released zinc would affect the activities of the enzymes involved in fibrogenesis and fibrinolysis in the liver. For instance, prolyl hydroxylase, a critical enzyme involved in the collagen synthesis, is a Fe^{2+} dependant enzyme (56). Zinc is an effective competitive inhibitor of this enzyme, replacing Fe^{2+} at the active site of the enzyme (57). In contrast, the catalytic activity of collagenases depends on zinc at the active center (58). Therefore, the availability of zinc in the liver affects the activities of the zinc-dependent or -sensitive enzymes involved in the fibrogenesis and fibrinolysis. Thus, it is interesting to know if MT functions in the process of hepatic fibrogenesis and fibrinolysis. In this context, we have undertaken a study to examine the role of MT in the reversal of toxicant-induced advanced liver fibrosis (52).

Mice treated with CCl_4 developed liver fibrosis. Upon cessation of CCl_4 dosing for 4 weeks, an almost complete reversal of the liver fibrosis was observed in the mice treated

with CCl_4 for 4 weeks. But the mice treated with CCl_4 for 8 weeks displayed an irreversible liver fibrosis. MT levels in the liver treated with CCl_4 for 8 weeks significantly decreased. Thus, the reversibility of liver fibrosis is related to the length of CCl_4 exposure and the presence of MT in the liver; the longer exposure the less reversibility, and the lack of MT in the liver makes the CCl_4-induced fibrosis persist after cessation of the exposure (52).

We applied a gene therapy approach using an adenoviral delivery system to deliver a human MT-II gene (Ad5-MT) into the liver through intravenous injection. The adenoviral delivery system efficiently increased MT expression in the liver three days after the injection in mice with irreversible liver fibrosis, leading to a significant fibrinolysis. In addition, MT gene therapy also causes hepatic cell regeneration (52).

This gene therapy study derived from the herbogenomics approach thus demonstrates that the reversibility of CCl_4-induced liver fibrosis was related to hepatic MT concentrations. MT was inducible in the liver by CCl_4; the reversal of CCl_4-induced liver fibrosis was accompanied by the spending of MT. High levels of MT at the time of cessation of CCl_4 dosing would predict a reversible fibrosis and low levels would be associated with a less reversible fibrosis. MT gene therapy made the irreversible liver fibrosis become reversible, which was shown to activate collagenases (predominately matrix metalloprotein-13 in the mouse liver) that would be highly responsible for the recovery of the irreversible fibrosis (52). In addition, the enhanced hepatocyte regeneration would also make a significant contribution to the MT gene therapy-induced recovery (52). Importantly, this gene therapy study strongly suggests the therapeutic potential of MT for patients with advanced liver fibrosis.

Conclusions and Perspectives. Herbogenomics is a novel analytical procedure for understanding the therapeutic effect and toxicity of herbal medicine preparation on certain disease progression through a profiling of the affected genomic alteration, therefore, providing mechanistic insights into that action of a particular herbal medicine preparation. There are many unique and effective TCM preparations that have proven on the evidence basis high efficacy in the treatment of chronic diseases and metabolic syndromes. The introduction of these effective TCM preparations to the Western world currently requires identification of active ingredients in these preparations, which is a very difficult undertaking. The application of herbogenomics will help in understanding the cellular and molecular targets on which TCM preparations act, thereby scientifically validating the therapeutic effect of TCM. This process will not only improve our current understanding of TCM in the modern medicine era, but also further explore the application of TCM in the discovery and development of new drugs and molecular therapies for diseases responsive to TCM treatment. Herbogenomics would thus provide a novel tool for the validation of TCM therapeutic efficacy and the enrichment of modern medicine.

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